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(54) Title: METHOD FOR THE CONTROL OF INFESTATIONS OF HONEYBEE COLONIES

(57) **Abstract**

The present invention concerns a method for the control of various diseases in bee-hives by applying to the bee-hives an effective amount of an essential oil in a slow-release formulation whereby the term oils embraces but is not limited to oils extractable from plant or the essential component thereof such as monoterpenes like menthol, geraniol, thymol, myrcene, citral, limonene, carene, camphor, eugenol, or cineol (eucalyptol); natural oils like lemon oil, eucalyptus oil, or neem oil; or organic acids like formic acid, acetic acid or oxalic acid. Most preferred are monoterpenes like thymol or menthol. Most preferred is thymol.

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METHOD FOR THE CONTROL OF INFESTATIONS OF HONEYBEE COLONIES

The present invention concerns a method for the control of acarid, lepidopteran, fungal, and bacterial infestations of honeybee colonies, and infestation controlling compositions suitable for such purpose.

Varroasis is an infestation of European honeybee colonies (Apis mellifera) with the ecto-parasitic mites belonging to the order of acaricides (Varroa jacobsoni).

The Varroa mite originated in Asia and from there has spread to virtually all countries where beekeeping with A. mellifera is practised.

In addition, other acarine infestations such as those of Acarapis woodii (Tracheal mite) and Tropilaelaps clareae; lepidopteran infestations such as those of the Greater and Lesser wax moths, Galleria mellonella and Achroia grisella respectively; dipteran infestation such as that of Braula caeca; fungal infection such as that of Chalk Brood, Ascosphaera apis and bacterial infections such as those of the American and the European Foulbroods, Bacillus larvae and Melissococcus pluton respectively, can cause significant damage to honeybee colony health.

The Varroa mites feed of the haemolymph of the developing bees and adults alike and can result in stunted growth of bees, infection and death of bee colonies. Indeed varroa infestation is the most serious threat to beekeeping world-wide today.

Various treatments are available for the control of this disease. However, in certain regions of Southern Europe, the Varroa mite has developed a resistance to the active ingredient of some of these various treatments, notably to products based on the pyrethroids flumethrin, fluvalinate and acrinathrin, but also to coumaphos, amitraz, malathion, cymiazole hydrochloride, chlorfenvinphos, bromopropylate, fenpyroximate, and related molecules.

It has now been found that the various diseases in bee-hives can be efficiently controlled by applying to the bee-hives an effective amount of an essential oil in a slow-release formulation.

Accordingly, the present invention provides for a method for controlling acarid, lepidopteran, fungal and bacterial infestations in colonies of honeybees, which comprises the application of an effective amount of an essential oil in a slow-release formulation to the locus of honeybee colonies.

For the purpose of this invention, the locus of colonies of honeybees is usually understood, but not limited, to bee-hives, or similar containers where colonies of bees build their breeding places and store food reserves, such as honeycombs.

An essential oil is understood to comprise oils extractable from plant or the essential component thereof which sometimes happens to be solid, and organic acids. Examples for essential oils are monoterpenes like menthol, geraniol, thymol, myrcene, citral, limonene, carene, camphor, eugenol, or cineol (eucalyptol); natural oils like lemon oil, eucalyptus oil, or neem oil; or organic acids like formic acid, acetic acid or oxalic acid. Most preferred are monoterpenes like thymol or menthol. Most preferred is thymol.

In this document, a slow-release formulation is meant to signify a formulation developed specifically to behave in a certain manner under defined conditions, characterised by a more regulated administration of active material over a given time-frame in comparison to raw active material. In particular, such formulations in connection with volatile essential oils, are capable of maintaining a constant vapour concentration of the essential oil in the atmosphere of an enclosed volume of a bee-habitat, i.e. a bee-hive. The proposed slow-release formulation is thus capable of releasing a regulated dose at a constant rate, preferably releasing a defined quantity of essential oil into a bee-hive over a chosen period of time, e.g. a period of 4-6 weeks, this period constituting at least one varroa mite reproductive cycle.

The active ingredient (essential oil or organic acid) is transported from the formulation via the vapour phase onto the target pest. In practice, the essential oil evaporates or sublimizes from the formulation in a regulated concentration into the atmosphere of the bee-environment (e.g. the beehive) and maintains the preselected concentration for at least a 4 to 6 weeks treatment period. Though the atmosphere in the bee environment is not hermetically sealed, the various pests in said environment can be effectively controlled by the release of the essential oil form the slow-release formulation. This also applies to the

pests affecting the bee larvae in the brood chambers, which by this method can be effectively protected.

The method of control of Varroa infestation according to the present invention is both, effective against pyrethroid resistant, as well as susceptible Varroa mite strains in bee-hives.

From the literature, the use of thymol in non-slow release formulation for attempting the control of Varroa infestations, is known. Also the use of thymol as a control agent against the bee tracheael mite Acarapis woodii had been suggested. However, the level of control achieved by hive treatment with raw material is low and at best, variable. With raw material the natural rate of evaporation from the crystal, powder or liquid forms, is largely dependent on ambient temperature, i.e. the dose administered to a beehive cannot be adequately regulated in the raw product form. For example, varroa treatment of bee colonies with thymol either requires multiple applications or high application doses, both of which methods causes honeybees to reject the hive. High doses are also toxic to the sensitive bee larvae.

The above disadvantages can be avoided by the method proposed by our invention. The new method is not only effective against varroa, but also controls all the other honeybee disease organisms aforementioned.

Examples of a slow-release formulation according to the present invention are gel formulations, either stand-alone or on bee-acceptable supports; impregnated materials; or polymer matrixes incorporating the above-mentioned essential oils or organic acids. Treatment of the infested bee colonies is preferably done in form of gel, or a gel strip, gel pellets, gel tablets, or even in a dispenser tray filled with any of the above forms, or any other type of slow-release dispersing system.

A slow-release matrix formulation may presented in any type of dispensing system, for example a shallow plastic tray dispenser with a hermetically sealing lid like a plastic or aluminium seal lid. The slow-release formulations used in the method according to the present invention, are prepared according to methods known per se to the worker in the art. However, the slow-release formulation, according to the present invention, are preferably

matrix-forming gels, which comprise the essential oil or organic acid in pure form or as a liquid formulation and a gel-forming amount of a thickener component and a bee-acceptable carrier material. This formulation type is suitable both for solid or liquid essential oils.

As thickener components, polymers of acrylic acid are in particular suitable. Commercial products available for this purpose are: carboxypolymethylenes, carboxyvinylpolymers, or carbomers like a CARBOPOL® (B.F.Goodrich Corp., Cleveland, Ohio). Other suitable thickener components are for instance carboxymethylcelluloses, polyvinyl acetate alcohols like a MOWIOL® (HOECHST AG, Frankfurt, Germany), longchain ammonium salts like BENTONE® (RHEOX GmbH, Leverkusen, Germany), hydrophilic polysaccharides like RHODOPOL® (RHONE POULENC, Paris) or KELZAN® (KELCO COMP. San Diego), or cellulose derivatives like TYLOSE (HOECHST AG, Frankfurt, Germany).

The preferred embodiment of slow-release formulations, according to the present invention comprises the active substance (essential oil or organic acid), the thickener agent with a cross-linking agent, and water, but not requiring any additional detergent to be present.

Typical concentrations of the thickener in the slow-release formulation, are from 0.01 to 1.5 % of the total weight, of the composition, preferably 0.1 to 1.0 %, e.g. 0.3 %, 0-4 % or 0.5 %. The actual concentration is however easily determinable by the worker in the art, according to the desired concentration of the essential oil in the bee-environment and the size of said environment.

The concentration of the essential oil in the overall composition is not critical, but reasonably is between 5 and 50 % of the total weight of the composition. Preferably, the concentration is between 10 % and 40 %, e.g. 20 %, 25 % or 30 %.

The mixture ratio of the essential oil to the thickener depends mainly on the amount of thickener required to transform the essential oil or organic acid and carrier mixture into a gel. In the given ranges of components, it is between 3:1 and 5000:1, preferably between 30:1 and 90:1, e.g. 40:1, 50:1, 60:1, or 70:1.

In a typical preparation of the slow-release-formulation the liquid or solid essential oil is added to a dispersion of the thickener like polyacrylic acid (e.g. CARBOPOL® EZ1) in water, and then the mixture is cross-linked by adding a suitable amount of a tertiary or secondary amine, like 0.01% to 2% of triethanolamine.

The cross-linking of the polyacrylic acid with an multifunctional amine gives the obtained gel a matrix like structure.

The obtained slow-release-formulation provides for releasing an effective amount of essential acid or organic acid over a defined period of treatment. Under effective amount we mean a sufficient amount of essential oil or organic acid to render at least 80 % infestation control of the parasite/pathogen without effecting significant mortality of the honeybee colony. The actual required and desirable concentration in the beehive can easily be determined by routine experimentation.

The new formulations so prepared provide the following advantageous properties:

1) The formulation comprises a regulated dose release of the active substance (essential oil or organic acid) into the beehive over a defined period of time and temperature range of 10-40 degrees Centigrade.

2. The formulation is highly effective in the control of pyrethroid-resistant as well as susceptible Varroa jacobsoni mites , parasitic on honeybees.

3. The formulation is also having efficacy against tracheal mite, A. woodii; the Greater and Lesser wax moth, Galleria mellonella and Achroia grisella respectively; the dipteran pest Braula caeca; fungal infections such as Chalk Brood, Ascosphaera apis, and bacterial infections such as those of the American and the European Foulbroods, Bacillus larvae and Melissococcus pluton, respectively.

The method of the present invention is preferably and ideally applied to bee populations or the bee environment in early spring or late summer, i.e. before or after the main honey flows but may not be restricted to these periods.

The slow-release formulation should be introduced to the interior of the beehive and left in place for a defined period of time, treatment duration being not shorter than 4 weeks and not longer than 6 weeks, at which time the formulation is to be removed from the hive.

Depending on the climatic region, it may be necessary to perform a second such treatment later in the year due to differences in reinfestation pressure.

Example 1: ***Preparation of a Thymol Slow-Release Formulation***

0.38 parts of CARBOPOL® EZ1 are slowly added to 73.86 parts of water with stirring. 25 parts of thymol are finely crushed and added to the stirred mixture. To the obtained dispersion, 0.76 parts of a 50 % aqueous solution of triethanolamine is added for cross-linking purposes, forming a gel.

The gel mixture is divided into 50 g portions and attributed to a shallow plastic tray dispenser. The surface of the tray is hermetically sealed with an aluminium or plastic foil lid. Alternatively, shallow aluminium trays may be filled with the gel and sealed with a plastic foil lid.

Example 2: ***Dispersion-Formulations***

Following the procedure of Example 1, slow-release formulations of solid essential oils are prepared having the following composition of matter:

Essential Oil	CARBOPOL® EZ1	Triethanolamine (50 % solution)	Water
Thymol 25 %	0.38 %	0.76 %	73.86 %
Thymol 20 %	0.38 %	0.76 %	78.86 %
Thymol 15 %	0.48 %	0.96 %	83.56 %
Thymol 10 %	0.48 %	0.96 %	88.56 %
Thymol 30 %	0.38 %	0.76 %	68.86 %
Thymol 35 %	0.38 %	0.76 %	63.86 %
Thymol 40 %	0.38 %	0.76 %	58.86 %
Camphor 25 %	0.38 %	0.76 %	73.86 %
Calcium oxalate to 25%	0.38 %	0.76 %	73.86 %

Example 3: ***Suspension-Formulations***

0.38 parts of CARBOPOL® EZ1 are slowly added to 73.86 parts of water with stirring. 25 parts of cineol (eucalyptol) are suspended using a high speed stirred until a homogeneous suspension is achieved. To the obtained suspension, 0.76 parts of a 50% aqueous solution of triethanolamine is added for cross-linking purposes, forming a gel.

The gel mixture is divided into 50 g portions and attributed to a shallow plastic tray dispenser. The surface of the tray is hermetically sealed with an aluminium or plastic foil lid. Alternatively, shallow aluminium trays may be filled with the gel and sealed with a plastic foil lid.

In a similar manner, the following formulations of liquid essential oils are prepared:

Essential oil	CARBOPOL® EZ1	Triethanolamine (50 % solution)	Water
Cineol 25 %	0.38 %	0.76 %	73.86 %
Limonene 25 %	0.38 %	0.76 %	73.86 %
Menthol 25 %*)	0.38 %	0.76 %	73.86 %
Neem-oil 25 %	0.38 %	0.76 %	73.86 %
Acetic acid 30 %	0.48 %	0.96 %	68.56 %
Formic acid 25 %	0.48 %	0.96 %	73.56 %

* process run at 40° C

Example 4: ***Biological Test***

Two trays of the 25 % thymol matrix slow-release formulation obtained from the preparation of Example 1, containing 50 grams of the slow-release gel each, are opened by removing the foil sealing lid and placed in a beehive on top of the broad frames. The test is started by placing the trays in the beehives, and is continued for 6 weeks. The average temperature inside and outside the beehives is recorded. The average temperature inside the beehive was 33 to 34°C, while outside the temperature varied between 17 and 35°C (night/day).

After 4 to 6 weeks, the infestation of the bee colony is assessed in comparison to an untreated control beehive by counting the total number of mites killed during the treatment and divided by the same total plus the number of mites falling after a final treatment with an acaricide, killing all surviving mites.

The results are as follows:

Treatment	Infestation at start	Infestation after 4 weeks	Reduction of Infestation in %
Thymol			
1 tray per hive	3240	1675	48.3%
2 trays per hive	2446	557	77.2%
Control	2873	2502	12.9%

Conclusion

The slow release formulation tested above is effective against Varroa jacobsoni infestations of bee hives as compared to Control (non-treated) colonies.

CLAIMS:

1. A method for controlling acarid, lepidopteran, fungal and bacterial infestations in colonies of honeybees, comprising the application of an effective amount of an essential oil or organic acid in a slow-release formulation to the locus of the honeybee colonies.

2. A method according to Claim 1 for controlling the pests of Varroa jacobsoni, Acarapis woodii and Tropilaelaps clareae; Galleria mellonella and Achroia grisella; Braula caeca; Ascosphaera apis; Bacillus larvae and Melissococcus pluton.

3. A method according to Claim 1 or 2 for the control of Varroa jacobsoni.

4. A method according to any one of Claims 1 to 2, wherein the concentration of the essential oil or organic acid is so chosen, that is controls the disease of varroa to a level of less than 20 % over a period of at least one varroa reproductive cycle.

5. A method according to Claim 4, wherein the concentration of the essential oil or organic acid in the environment of the bee colony is kept on an efficacious level over a 4-6 week treatment period.

6. A method according to any one of Claims 1 to 5, wherein the essential oil or organic acid is selected from monoterpenes, natural oils, or organic acids.

7. A method according to Claim 6, wherein the essential oil or organic acid is selected from menthol, geraniol, thymol, myrcene, citral, limonene, carene, camphor, eugenol, cineol, lemon oil, eucalyptus oil, neem oil, formic acid, acetic acid or oxalic acid, preferably thymol.

8. A slow-release gel formulation for the control of acarid, lepidopteran, fungal and bacterial infestations in colonies of honeybees, comprising an effective amount of an essential oil or organic acid in order to administer effective levels of said oil or acid during at least one reproductive cycle of varroa mites.

9. A formulation according to Claim 8, wherein the gel is in the form of a shallow tray dispenser with a hermetically sealing lid, gel strips, gel pellets, gel tablets, or a dispenser tray filled with the above forms.

10. A formulation according to Claims 8 or 9, wherein the essential oil or organic acid is selected from monoterpenes, natural oils, or organic acids.

INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER
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Minimum documentation searched (classification system followed by classification symbols)
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C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE WPI Derwent Publications Ltd., London, GB; AN 96-516754 XP002042391 EZHOVA: "Foot gel compsn. useful esp. for deodorant and fungicidal effects-contg. polymeric thickening agent and water, with additional ethanol, orange extract and mint essence , for cosmetics for long life footwear" see abstract & RU 2 056 832 A --- -/--	8-10

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Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE WPI Derwent Publications Ltd., London, GB; AN 88-349708 XP002042392 LION CORP: "Gel-type base material for fragrance compsn-obtd. by mixing nonionic cellulose deriv. with aq. gel obtd. by using carrageenan as gelling agent and locust beam gum as thickener " see abstract & JP 63 260 956 A	8-10
A	CHEMICAL ABSTRACTS, vol. 110, no. 9, 1989 Columbus, Ohio, US; abstract no. 71152, DENES ET AL.: "Control of Varroa jacobsoni on honeybees by thymol-containing compositions " XP002042389 see abstract & HU 45 691 A	
A	CHEMICAL ABSTRACTS, vol. 122, no. 1, 3 July 1995 Columbus, Ohio, US; abstract no. 416995, ESHBAH ET AL.: "Screening certain natural products and organic acids for controlling Varoa mite" XP002042390 see abstract & SHASHPA, vol. 1, no. 1, 1994, pages 53-62,	
A	SAMMATORO ET AL.: "Controlling tracheal mites (Acari: Tarsonemideae) in honey bees (Hymenoptera: Apidae)with vegetable oil" JOURNAL OF ECONOMIC ENTOMOLOGY, vol. 87, no. 4, 1994, page 910-916 XP002042386	
A	DELAPLANE: "Controlling Tracheal Mites (Acari: Tarsonemidae) in Colonies of Honey Bees (Hymenoptera: Apidae) with vegetable oil and menthol" JOURNAL OF ECONOMIC ENTOMOLOGY, vol. 85, no. 6, 1992, pages 2118-2124, XP002042387	

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INTERNATIONAL SEARCH REPORT

In. nal Application No
PCT/EP 97/03078

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CALDERONE N W ET AL: "PLANT EXTRACTS FOR CONTROL OF THE PARASITIC MITE VARROA JACOBSONI (ACARI: VARROIDAE) IN COLONIES OF THE WESTERN HONEY BEE(HYMENOPTERA: APIDAE)" JOURNAL OF ECONOMIC ENTOMOLOGY, vol. 88, no. 5, 1 October 1995, pages 1211-1215, XP000549665 -----	

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